February 8, 1938.

Dear Doctor Doan:

At last I got the hearth brush wrapped and sent to you, and a few days ago sent the Alice in Wonderland which I hope very much Elizabeth will enjoy.

You said you would be interested to find out what we had learned from the new study of polysaccharides. It has landed us in a bog because we have been using all kinds of controls which do almost the same thing. We made as our standard 10 mgms. of polysaccharide in 5 cc. saline. When you inject the 5 cc. saline alone, neutrophiles are called into the peritoneal cavity and in 24 hours they are abundantly phagocytized by the monocytes in the milk spots. Glucose gives an even more extreme reaction in this regard. The only difference that we have so far found with the polysaccharide, both from the tubercle bacillus and from the pneumococcus, is that they call so many young cells from the bone marrow that in 4 hours that they have actually exceeded in number the mature neutrophiles in the blood stream. This indicates that the polysaccharide acts like the saline only more intensely. With Type I polysaccharide from the pneumococcus we had also some stimulus of monocytes, in addition to the effect on the neutrophilic leucocytes. In one animal with Type III pneumococcus polysaccharide the action on monocytes was even more marked but the second animal, killed this morning, did not confirm this. Thus, as far as we have gone, almost anything put into the peritoneal cavity will call leucocytes from the blood stream and once out into the peritoneal cavity, they are apparently cleared away by the phagocytes. We have a new suggestion on the fixed technique which came from Mrs. Fuller in our department and has been worked out by Doctor Joyner. It is to fix blood films, films of peritoneal exudate, and films of omentum immediately in dioxane 25 parts and methyl (absolute) alcohol 75 parts for la minutes. After that, proceed at once with the Wright stain. The advantage of the technique is that the dioxane fixes all basophilic granules, so that the basophiles show up very clearly in the Wright stain. Doctor Joyner uses for blood films the slide method and makes the film by taking the drop on a half slide and inverting it crossways on a whole slide, and then drawing them apart quickly as soon as the blood has ceased spreading. He says as he draws the small slide across he lifts very slightly. At any rate, he gets very few smudges. I think you would be interested to try this technique.

One more point has come out in our studies of the polysaccharides. We introduced the tuberculopolysaccharide in saline without attempting to neutralize it. The polysaccharides from the pneumococcus, on the other hand, have been given as the sodium salt at a pH of 7.2 to 7.3 and the effects have been just the same. In following the fixed films, I am very greatly impressed with the importance of the right pH and I wonder

whether a slight acidity may not intensify an appearance of basophilia in stained cells. One more point in technique. Slides of bone marrow prepared by the simple touch technique and fixed in dioxane-methyl alcohol, as described above, give the most beautiful specimens of bone marrow we have ever seen. The basophiles stand out more sharply and it is possible to make, I think, quite accurate counts. The counts can be made with great rapidity because by the touch technique the cells are all in small, isolated foci.

You will be interested to know that Doctor Cunningham is coming down this week, Saturday, to have dinner with me. He planned to come last week but unfortunately had an attack of bronchitis. He seems to be all right now and I hope will be able to come. I long to hear how he is getting along at Albany.

I am so well that I have entirely forgotten that I was ever sick.

My best to the laboratory.

Cordially yours,

66 Florence R. Sabin.

Doctor Charles A. Doan, Kinsman Hall. Ohio State University, School of Medicine, Columbus, Ohio.